

US EPA ARCHIVE DOCUMENT

011337

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DATA EVALUATION RECORD

STUDY TYPE: Acute Mammalian Neurotoxicity - rat (81-8)

SHAUGHNESSY NO./TOX. CHEM. NO.: 128501/893C

ACCESSION NO./MRID NO.: 431323-01 (sulfosate); 430133-01 thru -
05 for positive controls; 430301-01 for
historical control data

DP BARCODE/SUBMISSION NO.: D200555, D200557, D200558, D200561,
D201511, D201514, D194075, D194071

TEST MATERIAL: Glyphosate Trimesium

SYNONYMS: Sulfosate

STUDY NUMBER(S): AR5425

REPORT NUMBER: CTL/P/3813

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory,
Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Glyphosate Trimesium: Acute Neurotoxicity
Study in Rats

AUTHOR(S): S. A. Horner

REPORT ISSUED: 2/15/93

CONCLUSION: Glyphosate Trimesium was tested in an acute neurotoxicity study in male and female Alderley Park AlpK:APfSD rats. Ten rats/sex were tested at each dose level, one time by gavage at 1 ml/100 g body weight. The following doses were tested: 0, 30, 100 or 300 mg/kg. Positive control data were provided.

At 300 mg/kg, the following effects were observed: death (2 on day 1); clinical signs of toxicity (ptosis (day 1), decreased activity (days 1-2), reduced splay reflex (days 1-4), upward curvature of the spine (days 1-5), chromodacryorrhea (days 1-3), shaking (days 1-3), sides pinched in (days 1-4), signs of urinary incontinence (day 1), irregular breathing (day 1), hunched posture (days 1-7), abnormal or staggering gait (days 2-7) and staining around the nose, in some cases up to days 4-7);

reduction in bodyweight in males on days 8 (9.5% less) and 15 (5.4% less); up to a 75.9% reduction in food consumption in males; increase in time to tail flick (273 - 281% of controls, 1-2 hours after dosing on day 1); reduction in landing foot splay (77 - 83% of controls, 1-2 hours post dosing on day 1); reductions in forelimb grip strength (82 - 85% of controls, 1-2 hours post-dosing on day 1); reduction in hindlimb grip strength (82% of controls, day 1) and reduction in motor activity (5.9 - 48.4% of controls, first hour after dosing on day 1). The results of the latter screening battery for neurotoxicity were not apparent on days 8 or 15 post-dosing, indicating that the effects were reversible. There was no microscopic evidence of neurotoxicity. No effects were observed at dose levels of 100 mg/kg or below.

The NOEL is 100 mg/kg and the LEL is 300 mg/kg based on death, clinical signs of toxicity, reduction in bodyweight and food consumption and effects on time to tail flick, landing foot splay, forelimb grip strength, hindlimb grip strength and motor activity during the first day after dosing. These were reversible by day 8. There was no microscopic evidence of neurotoxicity. There were no indications of neurotoxicity below at lethal dose.

This study is classified as Core Guideline and satisfies the guideline requirements for an acute mammalian neurotoxicity study in the rat (81-8).

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: N-(phosphono-methyl) glycine, sulfonium salt

Description: Amber yellow liquid

Batch #(s): F47 D7534/36 CTL Y06380/036

Purity: 59.4%

Source: ICI Agrochemicals

Vehicle (if applicable): Deionized water

Positive Control(s): chlordiazepoxide hydrochloride, morphine sulfate, amphetamine sulphate, chlorpromazine hydrochloride, trimethyltin chloride and acrylamide

2. Test Animals

Species and Strain (sexes): Male and female
Alpk:APfSD rats

Age: 35 days old upon arrival; 42 - 49 days old at start of test.

Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

- a. Preparation of Dose Levels: The test substance was weighed out for each dose level and an appropriate amount of the vehicle was added. Samples of each preparation were analyzed prior to the start of dosing in order to verify the concentrations desired. The chemical stability of glyphosate trimesium in deionized water was determined after a 7 day period. The homogeneity of the test chemical was not determined because the formulations were solutions.
- b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results of studies previously performed in the laboratory.
- c. Animal Assignment and Dose Levels: Rats were dosed one time by gavage at 1 ml/100 g body weight.

Test Group	Dose Administered	male	female
	mg/kg		
Control	0	10	10
1	30	10	10
2	100	10	10
3	300	10	10

*Five animals/sex from each group were designated for terminal neuropathology.

- d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study and daily during the study for clinical signs of toxicity and mortality.
- e. Body Weight Determinations: Bodyweights were recorded on day -1, immediately prior to dosing, 1-2 hours after dosing and on days 8 and 15.
- f. Food and/or Water Consumption: Food consumption was measured continuously throughout the study and calculated on a weekly basis.
- g. Functional Observational Battery: The report stated that "detailed clinical observations ...and quantitative assessments of landing foot splay, sensory perception (tail flick test) and muscle weakness (fore and hindlimb grip strength) were made on day -1, on day 1 (at 1 to 2 hours after

dosing), and on days 8 and 15. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour etc; reactivity to stimuli; changes in level of arousal; sensorimotor responses; [and] alterations in respiration. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition noted (slight, moderate or extreme) where appropriate. This included the recording of no abnormalities detected."

- h. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested on days -1, 1, 8 and 15. The report stated that "each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimize disturbances."
- i. Neuropathology: Any rat requiring euthanasia during the study and up to 5 rats/sex/group terminated at the end of the study were anesthetized with halothane, exsanguinated and subjected to a full post mortem examination. The tissues listed below were removed and fixed in 10% neutral buffered formol saline. The brains were weighed and the length and width were recorded with calipers. Also, five other animals/sex/group were deeply anesthetized with sodium pentobarbitone and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length and width were recorded. The tissues from these latter groups were further microscopically examined. The neuropathological examination was

performed on the control and highest dose groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, and 5 micrometer thick sections were cut and stained with H & E stain. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax and 5 micrometer thick sections were also cut and stained with H & E. The remaining tissues were embedded in ARALDITE and semi-thin sections (1-2 micrometers) were cut and stained with toluidine blue. Samples of the spinal cord and peripheral nerves were also embedded in ARALDITE and semi-thin sections cut and stained with toluidine blue. An initial examination of the brain was conducted on 1 male and 1 female from the 300 mg/kg group. The brain was examined in the transverse plane at 12 levels. On the basis of this examination, the remaining 4 animals/sex from this group and 5 rats/sex from the control group were examined in the transverse plane at the following 6 levels: 2, 5, 6, 7, 8 and 9. The spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was also examined in the transverse plane. Spinal roots and the dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia were examined from the trigeminal nerve. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were also examined. In addition, samples of the gastrocnemius muscle were examined in the transverse plane.

The following tissues were removed and examined microscopically:

x	Brain
x	Gasserian ganglia
x	Vertebral column including spinal cord
x	Dorsal root ganglia including spinal roots
x	Gastrocnemius muscle
x	Sciatic nerve
x	Sural nerve
x	Tibial nerve

- j. Statistical Analyses: Day 1 bodyweights, functional observational battery data (day -1 measurements), brain measurements on method of kill and the replicate structure of the study design were analyzed by analysis of covariance. Motor activity measurements for each 5 minute period and overall (minutes 1-50), weekly food consumption and replicate structure of the study design were all analyzed by analysis of variance. Least squares means for each group were calculated. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

1. Dosage Preparation: The concentration analyses revealed that the mean achieved concentrations were within 3% of the nominal concentrations (97.7, 98.0 and 101.7% of the the nominal concentrations for the 3.00, 10.0 and 30.0 mg/ml concentrations, respectively). The 3.0 and 30.0 mg/ml formulations were stable for a period of 7 days, which covered the period of use during the study (99.0% and 94.8% of the initial concentrations for the 2.95 mg/ml and the 31.8 mg/ml solutions, respectively).
2. General Clinical Signs of Toxicity and Mortality: There were two unscheduled deaths in the study. These were considered to be due to treatment with the chemical. Two high dose animals died, one male and one female. The male died 7-8 hours after dosing on day 1 and the female was killed in extremis approximately 6 hours after dosing on day 1. Clinical signs of toxicity for both animals were observed approximately 1-2 hours after dosing. These included, ptosis, decreased activity, reduced splay reflex and upward curvature of the spine in both animals; shaking in the male and reduced splay index in the female. In addition, approximately 6 hours after dosing, chromodacryorrhea, shaking, sides pinched in, signs of urinary incontinence and irregular breathing were also observed in the female. For humane reasons the animal was sacrificed.
3. Body Weight Determinations: In males, a statistically significant reduction in bodyweight was observed on days 8 and 15 at the high dose level when compared to the control group. This decrease was approximately

9.5% less than the control value at day 8 and 5.4% less than the control value at day 15. High dose females also had reductions in body weight but they were not statistically significant at either time point. No effects were observed at the lower dose levels in either sex. Thus, in males, there was a borderline effect on bodyweights at the high dose and in females there was no effect on bodyweights at any dose level. The following table summarizes body weights.

Intergroup Comparison of Bodyweights

Dose Level of Glyphosate Trimesium (mg/kg)

Day	0	30	100	300
Males				
1	149.4 ± 15.3	147.9 ± 14.6	146.7 ± 14.5	146.0 ± 16.3
8	222.0 ± 20.9	223.5 ± 17.9	221.3 ± 18.8	201.1** ± 21.0 (90.5%)
15	270.9 ± 22.3	274.6 ± 19.4	274.2 ± 21.1	256.4* ± 23.1 (94.6%)
Females				
1	126.2 ± 7.5	125.8 ± 7.6	127.6 ± 9.6	125.7 ± 11.1
8	171.1 ± 7.6	170.1 ± 8.8	172.3 ± 11.1	166.7 ± 13.6 (97.4%)
15	193.1 ± 7.5	190.7 ± 12.8	194.2 ± 12.6	184.8 ± 12.3 (95.7%)

*Significantly different from control (p < 0.05)

**Significantly different from control (p < 0.01)

() = % of control value

4. Food and/or Water Consumption: During week 1, reduced food consumption was observed in both sexes of the high dose group when compared to controls. Food consumption after that time was comparable to the control group. No treatment-related effects were observed in the lower dose groups. The following table taken directly from the report summarizes food consumption.

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Food Consumption (g/Rat/Day)

Week	Dose Levels (mg/kg)			
	0	30	100	300
Males				
1	25.7	25.4	25.5	19.5* (75.9%)
2	27.3	27.7	27.7	27.1
Females				
1	20.6	20.5	21.1	18.7* (90.8%)
2	20.5	20.0	20.7	19.7

*Statistically significant (p < 0.05)

5. Functional Observational Battery:

Clinical Observations: At the high dose, clinical signs were observed in both sexes. These were generally observed between 1-2 hours post dosing on day 1. Some signs continued to days 4-7. Recovery from the majority of the signs was observed within the first 24 to 48 hours post dosing. The signs included: ptosis, decreased activity, shaking, hunched posture, upward curvature of the spine, reduced splay reflex, sides pinched in and labored or irregular breathing. The report stated that abnormal, staggering gait was recorded from day 2 for 3 males and staining around the nose was also observed on day 2 for several animals. In addition, for several animals, abnormal gait, hunched posture, sides pinched in, upward curvature of the spine and reduced splay index were still apparent on days 4-7. No clinical signs were observed at any of the other dose levels. The following tables taken directly from the report summarize selected clinical signs of toxicity.

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Males	0	30	100	300
Abnormal gait				
# Observations				11
# Animals				3
Days (from - to)				2-7
Activity decreased				
Observations				13
# Animals				8
Days (from - to)				1-2
Labored breathing				
Observations				2
# Animals				2
Days (from - to)				2-3
Chromodacryorrhea				
Observations				3
# Animals				2
Days (from - to)				2-3
Hunched				
Observations				7
# Animals				4
Days (from - to)				1-7
Ptosis				
Observations				9
# Animals				9
Days (from - to)				1-1
Shaking				
Observations				11
# Animals				8
Days (from - to)				1-3
Sides pinched in				
Observations				8
# Animals				3
Days (from - to)				1-4
Reduced splay index				
Observations		6		6
# Animals		1		5
Days (from - to)		8-15		1-2
Stains around nose				
Observations				5
# Animals				4
Days (from - to)				2-3

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Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Males	0	30	100	300
Upward curvature of spine				
Observations				12
# Animals				7
Days (from - to)				1-5

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Females	0	30	100	300
Activity decreased				
Observations				5
# Animals				5
Days (from - to)				1-1
Breathing irregular				
Observations				2
# Animals				2
Days (from - to)				1-1
Ptosis				
Observations				5
# Animals				5
Days (from - to)				1-1
Shaking				
Observations				7
# Animals				5
Days (from - to)				1-3
Sides pinched in				
Observations				3
# Animals				2
Days (from - to)				1-3
Reduced splay index				
Observations		4		7
# Animals		1		4
Days (from - to)		1-4		1-4
Stains around nose				
Observations				2
# Animals				1
Days (from - to)				2-3

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Females	0	30	100	300
Upward curvature of spine				
Observations				10
# Animals				4
Days (from - to)				1-4

Tail Flick Response: In the high dose group, a statistically significant increase in time to tail flick was observed at 1-2 hours after dosing on day 1 for both sexes when compared with controls. By day 8, the time to tail flick response was slightly higher than controls in males, although not statistically significant. These animals had recovered by day 15. No treatment-related differences were observed for the lower dose groups. The following table taken directly from the report summarizes the most significant findings.

Intergroup Comparison of the Time to Tail Flick

		Dose Level (mg/kg)			
Day		0	30	100	300
Males					
-1		7.4 ± 5.0	8.3 ± 5.3	7.8 ± 5.4	6.4 ± 3.7
1		5.6 ± 2.4	4.9 ± 1.7	6.5 ± 3.1	15.3 ± 5.9
A.M. ^a		5.7	4.6	6.4	15.7**
8		7.2 ± 3.4	7.6 ± 5.5	6.5 ± 2.8	10.9 ± 6.5
A.M.		7.2	7.5	6.4	11.1
15		5.3 ± 5.4	6.9 ± 5.7	4.0 ± 3.1	4.2 ± 1.5
A.M.		5.3	6.8	4.0	4.0
Females					
-1		8.9 ± 6.3	7.3 ± 5.3	10.6 ± 6.6	7.0 ± 4.6
1		5.3 ± 2.7	6.6 ± 4.6	6.6 ± 5.6	14.9 ± 5.5
A.M.		5.3	6.6	6.7	14.8**
8		4.3 ± 1.9	6.9 ± 4.7	5.8 ± 3.7	5.2 ± 3.4
A.M.		4.3	7.1	5.6	5.4
15		4.9 ± 2.6	4.8 ± 4.0	4.3 ± 2.3	6.6 ± 5.2
A.M.		4.8	4.9	4.2	6.5

**Statistically significant (p < 0.01)

^aA.M. = Adjusted mean.

Landing Foot Splay: A statistically significant reduction in landing foot splay was observed at 1-2 hours post dosing on day 1 in high dose males and in females in all dose groups. Considering that for all other tests there were no effects in the 2 lower dose groups in either males or females and that the low and mid-dose males were not affected in this particular test, the observed effects in females at the two lower dose levels are probably not biologically significant. By day 8, landing foot splay was comparable to the controls in all groups. The following table, taken directly from the report, summarizes landing foot splay measurements.

Intergroup Comparison of Landing Foot Splay (mm)

Day	Dose Level (mg/kg)			
	0	30	100	300
Males				
-1	63.0 ± 14.6	64.5 ± 10.7	58.3 ± 19.0	64.0 ± 12.2
1	56.8 ± 11.5	58.2 ± 11.7	60.0 ± 11.8	47.2 ± 10.0
A.M. ^a	56.6	57.5	61.5	46.6*
8	61.5 ± 11.9	62.5 ± 16.2	68.8 ± 13.7	69.1 ± 14.8
A.M.	61.4	62.1	69.7	68.5
15	65.3 ± 13.1	64.8 ± 7.3	73.3 ± 14.6	73.3 ± 15.2
A.M.	65.2	64.1	74.9	72.4
Females				
-1	58.0 ± 9.2	61.3 ± 13.9	61.0 ± 17.5	61.7 ± 11.1
1	58.5 ± 11.3	50.7 ± 11.0	43.0 ± 10.3	45.0 ± 7.1
A.M.	59.6	50.3*	42.8**	44.5**
8	61.7 ± 11.5	61.5 ± 13.3	54.2 ± 14.8	57.0 ± 11.9
A.M.	62.7	61.1	53.9	56.1
15	56.0 ± 5.7	61.3 ± 12.6	56.9 ± 13.7	61.1 ± 14.1
A.M.	56.7	61.0	56.7	60.6

^aA.M. = adjusted mean

*Statistically significant (p < 0.05)

**Statistically significant (p < 0.01)

Grip Strength: Statistically significant reductions in forelimb grip strength was observed 1-2 hours post-dosing on day 1 in high dose males. Forelimb and hindlimb grip strength were significantly reduced in high dose females on day 1 as well. On day 15,

significant reductions in forelimb grip strength in 100 mg/kg males and in hindlimb grip strength in 30 or 300 mg/kg females were observed. These are not considered to be biologically significant because the changes are not consistent across dose levels, sex or time of observation. The following tables taken directly from the report summarize the results.

Intergroup Comparison of Forelimb Grip Strength (g)

Day	Dose Level (mg/kg)			
	0	30	100	300
Males				
-1	610 ± 95	669 ± 83	599 ± 117	652 ± 75
1	801 ± 130	791 ± 160	720 ± 150	658 ± 80
A.M. ^a	818	763	745	643*
8	831 ± 207	876 ± 237	729 ± 153	787 ± 213
A.M.	835	868	735	767
15	1228 ± 210	1144 ± 217	1053 ± 174	1126 ± 112
A.M.	1242	1120	1074*	1120
Females				
-1	641 ± 153	586 ± 98	637 ± 184	652 ± 144
1	771 ± 110	776 ± 99	691 ± 94	659 ± 129
A.M.	766	793	688	649**
8	814 ± 147	844 ± 171	798 ± 179	782 ± 206
A.M.	812	853	797	758
15	1158 ± 84	1123 ± 128	1106 ± 93	1084 ± 160
A.M.	1158	1120	1106	1086

*Statistically significant (p < 0.05)

**Statistically significant (p < 0.01)

^aA.M. = adjusted mean

Intergroup Comparison of Hindlimb Grip Strength (g)

Day	Dose Level (mg/kg)			
	0	30	100	300
Males				
-1	431 ± 150	420 ± 110	397 ± 77	401 ± 73
1	483 ± 76	498 ± 138	438 ± 62	449 ± 89
A.M. ^a	477	495	442	452
8	616 ± 104	643 ± 79	596 ± 90	631 ± 135
A.M.	618	643	594	628
15	670 ± 80	642 ± 205	662 ± 91	716 ± 150
A.M.	674	644	658	718
Females				
-1	488 ± 87	428 ± 56	487 ± 109	442 ± 54
1	484 ± 71	461 ± 105	486 ± 66	395 ± 102
A.M.	479	466	482	398*
8	635 ± 112	644 ± 97	566 ± 116	629 ± 73
A.M.	632	648	563	632
15	743 ± 144	579 ± 113	723 ± 108	610 ± 86
A.M.	737	586**	717	619*

*Statistically significant (p < 0.05)

**Statistically significant (p < 0.01)

^aA.M. = Adjusted mean

- Motor Activity:** In the high dose group, motor activity was significantly reduced during the first hour after dosing on day 1 when compared to controls. For males, the difference was especially observed during the first 10 minutes and for females, the difference was especially observed during the first 5 minutes. During minutes 6 - 10, motor activity for females was reduced when compared to controls, but not significantly so. No treatment-related differences were observed in the other dose groups. The following table taken directly from the report summarizes selected values for motor activity.

Intergroup Comparison of Motor Activity (Movements/Animal)

Day Minutes	Dose Level (mg/kg)			
	0	30	100	300
Males				
Day -1				
Min. 1-5	65.9 ± 12.1	72.1 ± 11.7	76.9 ± 8.2	68.7 ± 11.0
Min. 6-10	56.4 ± 27.9	51.9 ± 20.7	60.9 ± 15.9	50.6 ± 18.3
Day 1				
Min. 1-5	59.3 ± 13.6	66.3 ± 15.8	64.3 ± 6.7	3.5 ± 4.1**
Min. 6-10	22.6 ± 22.9	32.3 ± 25.3	21.9 ± 16.9	1.7 ± 3.0*
Min. 11-15	5.8 ± 8.5	12.4 ± 26.3	3.9 ± 11.3	0.9 ± 1.7
Min. 16-20	0.6 ± 1.1	4.1 ± 10.9	0.4 ± 1.0	1.7 ± 2.8
Min. 21-25	0.5 ± 1.0	0.3 ± 0.7	0.0 ± 0.0	5.0 ± 9.3*
Min. 26-30	0.2 ± 0.4	0.9 ± 2.2	0.9 ± 1.4	2.5 ± 4.4
Min. 46-50	0.0 ± 0.0	5.5 ± 8.3**	0.8 ± 1.3	0.5 ± 1.1
Min. 1-50	100.0 ± 54.5	127.8 ± 68.1	95.2 ± 28.8	17.7 ± 15.9**
Day 8				
Min. 1-5	74.1 ± 8.9	73.9 ± 7.9	74.4 ± 7.9	69.7 ± 23.0
Min. 6-10	64.4 ± 10.2	54.9 ± 22.6	66.9 ± 13.0	47.1 ± 22.2*
Min. 46-50	5.0 ± 9.4	10.7 ± 21.3	10.7 ± 19.4	31.9 ± 26.7**
Min. 1-50	298.5 ± 78.6	249.1 ± 76.9	280.2 ± 116.1	300.1 ± 140.6
Day 15				
Min. 1-5	73.3 ± 14.4	75.5 ± 11.7	79.7 ± 7.2	73.0 ± 15.5
Min. 1-50	508.4 ± 158.9	508.8 ± 179.7	493.8 ± 191.6	506.9 ± 142.0
Females				
Day -1				
Min. 1-5	66.7 ± 12.8	67.1 ± 10.1	65.6 ± 11.8	65.7 ± 10.3
Min. 6-10	59.0 ± 12.7	63.7 ± 10.1	47.6 ± 20.0	56.9 ± 15.9
Day 1				
Min. 1-5	62.6 ± 11.3	71.1 ± 9.7	68.8 ± 12.8	30.3 ± 31.8**
Min. 6-10	36.1 ± 30.0	51.9 ± 21.3	44.9 ± 25.0	25.0 ± 22.2
Min. 11-15	21.8 ± 28.5	29.8 ± 28.0	28.6 ± 31.1	16.9 ± 25.4
Min. 16-20	4.8 ± 10.1	23.2 ± 25.5*	11.7 ± 16.5	16.5 ± 22.7
Min. 21-25	2.1 ± 3.0	22.1 ± 28.4*	15.2 ± 18.7	14.0 ± 16.8
Min. 26-30	5.4 ± 15.0	17.1 ± 24.1	16.0 ± 21.9	9.8 ± 10.9
Min. 36-40	7.2 ± 15.7	23.7 ± 26.1*	11.4 ± 12.3	6.4 ± 11.0
Min. 41-45	2.2 ± 5.3	31.6 ± 34.4*	12.3 ± 25.7	15.3 ± 25.5
Min. 1-50	154.0 ± 84.7	315.2 ± 161.0**	242.1 ± 150.2	150.9 ± 132.8
Day 8				
Min. 1-5	65.3 ± 7.0	64.4 ± 20.5	68.0 ± 11.5	66.3 ± 16.3
Min. 11-15	46.7 ± 24.1	62.5 ± 13.7*	49.5 ± 16.2	57.9 ± 8.0
Min. 1-50	544.7 ± 131.7	621.9 ± 121.6	543.6 ± 120.5	595.6 ± 125.7
Day 15				
Min. 1-5	70.3 ± 9.9	66.0 ± 17.0	71.8 ± 10.2	68.2 ± 10.5
Min. 1-50	565.8 ± 101.0	612.8 ± 164.7	585.1 ± 161.5	605.7 ± 126.0

7. Brain Measurements: No treatment-related differences were observed in brain weight, length or width at any dose level in either sex. The following table taken directly from the report summarizes the values.

Intergroup Comparison of Brain Measurements

Observation	Dose Level (mg/kg)			
	0	30	100	300
Males				
Brain Weight (g)	1.90	1.89	1.89	1.87
Brain Length (mm)	27.3	26.2	26.2	27.1
Brain Width (mm)	15.1	14.9	15.4	15.0
Females				
Brain Weight (g)	1.77	1.76	1.78	1.78
Brain Length (mm)	26.2	25.9	27.0	26.3
Brain Width (mm)	14.9	14.8	14.8	15.1

8. Neuropathology: Minimal nerve fiber degeneration was observed in the sciatic nerve of 1 high dose male. The degeneration was minimal and consisted of a single, small focus of Wallerian type degeneration. The authors of the report stated that "such occasional nerve fiber degeneration is an incidental feature of the peripheral nervous system of a number of strains of rat, including the Alderley Park strain. In this study, the single small focus of Wallerian type degeneration seen, in one fiber, was considered to be a spontaneous finding and incidental to treatment...". In light of historical control data (see table below), this lesion is not considered to be related to treatment. The following table taken directly from the report summarizes the results.

Intergroup Comparison of Microscopic Findings

Dose Level of Glyphosate Trimesium
(mg/kg)

	Males		Females	
	0	300	0	300
Animals on Study	10	10	10	10
Animals Completed	5	5	5	5
Sciatic Nerve Examined	5	5	5	5
No Abnormalities Detected	5	4	5	5
Nerve fibre degeneration (total)	0	1	0	0
minimal	0	1	0	0

In response to a question concerning these same type of lesions observed with another pesticide submitted by Zeneca and tested in the same laboratory, historical control data were submitted on these lesions. The following table summarizes these data.

Historical Control Incidence of Nerve Fiber Degeneration in Sciatic Nerve of Alderley Park Rats^a

Acute Oral Studies: N = 5 Rats/Sex/Group

Month/Year	Males	Females
May 1992	2	0
June 1992	0	0
July 1992	2	1
December 1992	1	1
February 1993	1	0

^aThe data refer to the Alpk:APfSD (Wistar-derived) strain of rat. Nerve fiber degeneration is defined as foci of either Wallerian type degeneration/axonal swellings and/or areas of demyelination. The grading of nerve fiber degeneration seen was minimal for all animals. A grading criteria of minimal represents one to several small foci of Wallerian type degeneration originating from 1-2 nerve fibers.

9. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance Statements were provided.

- C. DISCUSSION: This study was conducted according to the guidelines and is graded Core Guideline. It satisfies the regulatory requirements for an acute mammalian neurotoxicity study (81-8). Positive control data were provided under separate cover and are summarized in the Appendix. The NOEL is 100 mg/kg and the LEL is 300 mg/kg. Toxicity was observed at the highest dose level in both sexes (300 mg/kg).

There were positive results for the neurotoxicity screening battery on day 1. These effects had disappeared by day 8. Since the protocol does not call for observations in between days 1 and 8, it is not known how long these effects lasted. However, some of the clinical signs of toxicity that are similar to clinical signs of neurotoxicity persisted to day 7 (it is noted that in metabolism studies, this chemical is eliminated from the body within 1 to 5 days). In this case, the authors of the report stated that these effects were due to systemic toxicity and not neurotoxicity because the animals were tested at a dose level close to the LD₅₀. Since there were no microscopic indications of neurotoxicity, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity. It is noted here that the only change observed in the subchronic neurotoxicity study was a decrease in forelimb grip strength in high dose females at various time points. This was not seen in males and was not dose-related, although consistent at the high dose. No other differences were observed in the subchronic study. Therefore, if the results of the acute study are indicative of neurotoxicity, they are not validated by the results from the subchronic study. The positive control data submitted with these studies do not shed any light on how to interpret the data. Three of the positive control studies were terminated 1 hour after dosing and the other two were subchronic studies. In these, either the neurotoxic effects were different from the effects seen in this particular study or they appeared later in the study and not on the first day.

Appendix: Discussion of Positive Control Studies

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Pamela M. Hurley 5/11/94
Roger L. Gardner
5/26/94

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for
Assessment of Sensory Perception in the Rat

ACCESSION NO./MRID NO.: 430133-02

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Morphine sulphate

STUDY NUMBER: XR2287

REPORT NUMBER: CTL/P/3689

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington,
Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley
Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Assessment of Sensory Perception in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Morphine sulphate (99%) was tested as a positive control in the tail flick test in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 50, 75 or 100 mg/kg of the test material in deionized water at a volume of 1 ml/100g bodyweight. The tail flick response test for pain perception was conducted one hour after dosing.

At 100 mg/kg, an increase in the tail flick response time (219 - 234% of control time) was observed in both sexes. No clear treatment-related effects were observed at the lower dose levels.

The NOEL for tail flick time response is 75 mg/kg and the LEL is 100 mg/kg.

This study is acceptable as a positive control study for the laboratory in which it was conducted, for chemicals that induce an analgesic or soporific effect. As a general comment, the animals were examined in the tail flick test at one hour after dosing and not at any time afterwards (it is assumed that this is

because the analgesic effect of morphine does not last for a long time). For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD₅₀. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Morphine sulphate

Description: White solid

Batch #(s), Other #(s): CTL Ref. No. Y05725/005

Purity: 99% w/w

Source: Sigma Chemical Company

Vehicle (if applicable): deionized water

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats

Age: Between 5 and 8 weeks

Weight(s): 130-184g (males); 107-164g (females)

Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

3. Procedure:

- a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of deionized water.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

- b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.
- c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Admin- istered mg/kg	Main Study	
		male	female
Contr.	0	10	10
1	50	10	10
2	75	10	10
3	100	10	10

- d. Measurement of Tail Flick Response: The tail flick time of each animal was measured the day before dosing. Any animal with a response time of greater than 9.5 seconds was replaced. Tail flick time of each animal was again measured 1 hour following dosing. The report stated that "the test involved the application of a thermal stimulus to the tail and measurement of the latency to withdraw the tail. A cut-off time of 20 seconds was used."

No other measurements were conducted.

- e. Statistical Analyses: Time to tail flick was analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Tail Flick Response

The test chemical prolonged the tail flick response time in both sexes at the highest dose tested (100 mg/kg). There were no clear treatment-related effects at the lower dose levels. The following table, taken directly from the report summarizes the results.

Intergroup Comparison of Tail Flick Times (seconds)

Dose Level of Morphine Sulfate (mg/kg)

0	50	75	100
Males			
5.47 ± 2.42	7.31 ± 4.99	5.65 ± 2.28	12.83 ± 5.42**
Females			
4.58 ± 2.10	6.53 ± 3.31	5.28 ± 1.21	10.06 ± 4.67**

**Statistically significant (p < 0.01)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

- C. DISCUSSION: Since the purpose of this study was to show that the tail flick response test is valid in this test system, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that sensory perception in the rat can be measured using the tail flick test. The report stated that "the characteristic analgesic effect of morphine sulphate was demonstrated in this study through an increase in response times at 100 mg/kg."